solubilized preparation of rat myocardial adenylate cyclase, but results in a striking increase in sensitivity to norepinephrine. This degree of sensitivity approaches that observed in intact physiological preparations (19).

Phosphatidylinositol is normally present in heart tissue. It comprises about 3.5% of lipid-phosphorus in rat heart, 2.3% in sheep heart, and 7.9% in ox heart (20) or approximately 0.02 mg to 0.11 mg of lipid-phosphorus per g of heart muscle, fresh weight. It may be calculated that incubations with the particulate enzyme contained about 0.001 to 0.022 μg of phosphatidylinositol, values roughly comparable to the phosphatidylinositol required in the solubilized preparation as shown in Fig. 3.

Since competitive beta adrenergic blocking agents, such as dl-propranolol, abolish catecholamine activation of the enzyme in intact muscle and in particulate heart preparations (1, 3, 21), it would appear that beta adrenergic blockade would be a necessary prerequisite in considering the specificity of any in vitro system attempting to define the molecular nature of a beta adrenergic receptor. The data show that dl-propranolol abolished the activation of solubilized adenylate cyclase produced by norepinephrine in the presence of phosphatidylinositol.

Recently, Lefkowitz and Haber (22) isolated a partially purified cardiac beta receptor in a microsomal fraction of canine ventricle. They measured displacement of bound [3H]norepinephrine by unlabeled norepinephrine and determined that 50% displacement occurred at 1 x 10^-8 M norepinephrine. This figure is similar to that obtained for half-maximal activation of the solubilized adenylate cyclase by norepinephrine. The Lefkowitz and Haber preparation also seems to provide a useful system for determining the molecular components of the cardiac beta receptor.

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REFERENCES

Synthesis of Ovalbumin in a Rabbit Reticulocyte Cell-free System Programmed with Hen Oviduct Ribonucleic Acid*

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SUMMARY

Hen oviduct RNA can direct the synthesis of the major oviduct protein, ovalbumin, in a cell-free system derived from rabbit reticulocytes. The protein was identified by precipitation with anti-ovalbumin, migration on gel electrophoresis, and ion exchange chromatography of tryptic peptides. Ovalbumin messenger RNA activity appears to sediment in a sucrose gradient on the low molecular weight side of the 18 S ribosomal RNA.

Administration of estrogen to immature chicks leads to cytodifferentiation of the oviduct (1-3). Ovalbumin accounts for 50 to 60% of the total protein synthesized in the fully differentiated state (4), and its synthesis is dependent on continued administration of hormone (3, 5). Interest in our laboratory has been focused on detection and isolation of ovalbumin messenger RNA in order to understand better the events connected with steroid induction of protein synthesis and differentiation. We present here evidence that the rabbit reticulocyte lysate system, originally described by Adamson et al. (6), can synthesize ovalbumin when programmed with hen oviduct RNA.

Reticulocytes were prepared by the method of Evans and Lingel (7). Cells were lysed by addition of an equal volume of water, and cell debris was removed by centrifugation at 12,000 x g for 10 min. Lysate was generally prepared in large quantities and stored at liquid nitrogen temperature. Conditions for cell-free protein synthesis were those of Lockard and Lingel (8), except reactions contained phosphoenolpyruvate (5 mM) and pyruvate kinase (5 enzyme units per ml) instead of creatine phosphate and creatine phosphokinase, 50 μM nonradioactive leucine, 25 μCi per ml of [3,4-3H]leucine (Schwarz-Mann, specific activity 40 Ci per mmole), and no 2-mercaptoethanol. After incubation at 30° for 30 min, 100-μl aliquots of the reaction mixture were analyzed in duplicate for anti-ovalbumin-precipitable radioactivity using 5 μg of nonradioactive ovalbumin as carrier by the method of Palmiter et al. (9). Triton X-100 (0.66%) was included in the antibody reaction mixture, and the precipitate was washed three times with 10 mM sodium phosphate buffer, pH 7.5, containing 160 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, and 10 μM leucine. Parallel reactions were carried out in duplicate using 5 μg of bovine serum albumin and rabbit anti-ovalbumin.
TABLE I

<table>
<thead>
<tr>
<th>RNA added</th>
<th>Concentration µg/ml</th>
<th>Ouali-</th>
<th>Total</th>
<th>Percent-</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>albumin</td>
<td>protein</td>
<td>of total</td>
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<tr>
<td></td>
<td>µcpm/100 µg</td>
<td>anti-BSA</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>µcpm X 10^4/100 µg</td>
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</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>47</td>
<td>10.00</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Hen oviduct polysomal</td>
<td>10</td>
<td>15.50</td>
<td>0.15</td>
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</tr>
<tr>
<td>Fraction 1</td>
<td>40</td>
<td>8.50</td>
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<tr>
<td>Fraction 2</td>
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<td></td>
<td>480</td>
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<td>0.62</td>
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<td>7.22</td>
<td>3.02</td>
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<tr>
<td>Hen liver</td>
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<td>8.71</td>
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<td>6.67</td>
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<td>1160</td>
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<td></td>
<td>1250</td>
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</tbody>
</table>

*Non-specific radioactivity trapped by anti-BSA ranged from 85 to 140 cpm with an average of 110 cpm.

1 Fractions 1 and 2 correspond to those shown in Fig. 3. Tissue from the magnum portion of the oviduct of an actively laying hen was homogenized with a Dounce homogenizer in 9 volumes of polysome buffer (0.25 M Tris-HCl, pH 7.4, at 4°, 25 mM NaCl, 5 mM MgCl₂, containing 140 mM sucrose, 100 µg per ml of sodium heparin, 1% sodium deoxycholate, and 1% Triton X-100), and centrifuged at 27,000 X g for 5 min. Heavy polysomes were separated from lighter polysomes, monosomes, and the RNA was precipitated with 2 volumes of cold ethanol. After standing overnight at -20° RNA fractions were pelleted by centrifugation.

2 Total nucleic acid was extracted from hen oviduct and liver as follows: 20 g of tissue were homogenized in a Waring Blender for 1 min in 100 ml of ice-cold 0.5% SDS, 25 mM EDTA, 75 mM NaCl, pH 8.0, and 100 ml of freshly distilled phenol which was saturated with buffer and adjusted to pH 8.0. The phases were separated by centrifugation at 10,000 X g for 10 min. After a second extraction of the aqueous phase with phenol, nucleic acid was precipitated from the aqueous phase with 2 volumes of ethanol at -20°. The precipitate was redissolved in 0.01 M sodium acetate, pH 5.0, made 0.1 M sodium acetate, and precipitated five times. Hen oviduct and liver nucleic acid were generously provided by Mr. Morris Summers.

The abbreviations used are: BSA, bovine serum albumin; SDS, sodium dodecyl sulfate.

From the size of the ovalbumin molecule (387 amino acid residues (11)) one can calculate a minimum molecular weight of 350,000 for ovalbumin mRNA, and estimate a sedimentation coefficient of approximately 14 S (12). RNA was prepared from hen oviduct polysomes, and a fraction sedimenting between about 11 S and 17 S was tested in the cell-free protein-synthesizing system (Table I, Experiment 1). Increasing concentrations of RNA produced nearly linear increases in the percentage of total radioactivity precipitated by anti-ovalbumin. The 28 S ribosomal RNA species (Fraction 1 in Table I) failed to produce anti-ovalbumin precipitable radioactivity. To rule out the possibility...
that the absence of activity was due to inhibitory substances that interfere with mRNA detection, this fraction was tested in the presence of 11 to 17 S RNA (Experiment 1, Fractions 4 + 1). Some inhibition was observed, both in antibody-precipitable radioactivity and in percentage of total incorporation, but activity was not completely suppressed, indicating the absence of ovalbumin mRNA activity in the 28 S rRNA fraction. Experiment 2 shows that detection of ovalbumin mRNA activity does not require a highly purified RNA fraction. Total phenol-extracted nucleic acid from hen oviduct was active in directing the synthesis of anti-ovalbumin precipitated radioactivity, although hen liver nucleic acid obtained by the same procedure was inactive. A mixing experiment similar to that described above showed that the liver nucleic acid preparation partially suppressed the translation of ovalbumin mRNA, but not completely. To test whether

the reticulocyte lysate system could be saturated with ovalbumin mRNA, the concentration dependence of ovalbumin synthesis was investigated at high levels of oviduct nucleic acid. Experiment 3 shows that the system continued to give a linear response at nucleic acid concentrations as high as 1.2 mg per ml where the radioactivity in ovalbumin represented more than 10% of that in total protein.

Two experiments were performed to characterize the protein synthesized under the direction of oviduct RNA. The molecular size of the antibody-precipitable radioactivity was determined by SDS-acrylamide gel electrophoresis (9). When oviduct polysomal RNA was added to the cell-free system the major portion of radioactivity migrated in a single peak (Fig. 1B). The mobility of this peak was similar to that of [14C]ovalbumin prepared in oviduct explants (9), Fig. 1A). [3H]Ovalbumin was subjected to electrophoresis on a separate gel to avoid possible errors due to spillover of the 14C radioactivity into the 3H channel. To determine the mobility of the reaction product more accurately, 3H-antibody precipitate from a reaction containing total oviduct nucleic acid was subjected to electrophoresis with [14C]ovalbumin as an internal marker. The protein synthesized in the reticulocyte lysate had a slightly higher mobility (Fig. 1C). A possible explanation for this is that the ovalbumin molecule synthesized in the cell-free system lacks the carbohydrate moiety (mol wt approximately 1500 (11)). Fig. 1D shows that in the absence of oviduct RNA the reticulocyte lysate does not synthesize detectable amounts of protein having the electrophoretic mobility of ovalbumin.

In a second experiment [3H]anti-ovalbumin precipitate and authentic [14C]ovalbumin were digested with trypsin, and peptides were partially resolved by ion exchange column chromatography (Fig. 2A). The correspondence of each 3H peak with a
\(^{14}C\) peak further suggests the identity of the protein synthesized under the direction of oviduct RNA as ovalbumin. As a control, total \(^{3}H\) reticulocyte protein from a reaction mixture containing no oviduct RNA was combined with \(^{14}C\) ovalbumin and subjected to tryptic digestion and chromatography (Fig. 2B). The fact that \(^{3}H\) and \(^{14}C\) peaks do not coincide rules out the possibility that the correspondence seen in Fig. 2A is due to contamination by total protein.

In an initial attempt to purify ovalbumin mRNA and to estimate its molecular size, polysomal RNA was fractionated by sucrose gradient sedimentation and tested in the reticulocyte lysate system (Fig. 3). The fraction corresponding to the low molecular weight part of the 18 S rRNA peak had the highest specific activity, as would be expected from the predicted size of ovalbumin mRNA.

Relatively few eukaryotic mRNAs have been identified in a functional assay. Evidence has been presented that hemoglobin mRNAs from several species can be translated in a variety of protein-synthesizing systems (8, 15–17). The rabbit reticulocyte lysate system used by Lockard and Lingrel (8) was also used for the detection of a mouse immunoglobulin light chain by Stavnezer and Huang (18). Our results along with those mentioned above strongly argue against an absolute requirement for protein- or species-specific initiation factors.

The apparent ease with which we have been able to obtain ovalbumin in the reticulocyte lysate system suggests that it can be used for the assay of any number of specific mRNAs, provided that a technique such as immunoprecipitation is available for the isolation of the specific product. Our finding that a specific mRNA can be translated in the presence of total cellular nucleic acid may eliminate the necessity for isolating polysomes in order to measure the mRNA content of a tissue. The use of this assay should facilitate the isolation of ovalbumin mRNA in undegraded form and make possible the quantification of ovalbumin mRNA in various hormonal and developmental states of the oviduct.

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